Effect of Nitrate Esters of Androgen Steroids on the Succinate Oxidase and NADH-Cytochrome C Reductase Activity of Isolated Rat Liver Mitochondria

In parallel with the biochemical and physiological development, there has been an enormous expansion of interest in the synthesis and biological testing of substances related to the naturally occurring steroid hormones. This activity has yielded a large number of steroids and related compounds which mimic the biological activity of the natural hormones or act as antagonists.

Recently in our laboratories some nitrate esters of androgens were obtained; but we did not find in the literature any data concerning their biochemical effects. Our data would have great significance for biology and medicine[1].

In the present study, the effect of testosterone (T), testosterone-nitrate (TN), 5α-androstane-17β-ol-3-one (A), 5α-androstane-17β-ol-3-one nitrate (AN), 5α-androstane-3α-ol-17-one (B) and 5α-androstane-3α-ol-17-one nitrate (BN) on the succinate oxidase and NADH-cytochrome *c* reductase (SuOx respectively NCCR) activity of isolated rat liver mitochondria was investigated.

Materials and method. 168 adult white male rats, weighing 150 + 10 g were used. Liver mitochondria fractions were prepared according to the method of Hogeboom and Schneider[2]. Mitochondria obtained were resuspended in 0.33 *M* sucrose solution and were used for the determination of SuOx and NCCR activity[3,4]. NCCR activity was measured by using the 'frozen-thawed' mitochondrial preparation to facilitate the permeation of NADH[5]. Mitochondrial protein concentration was determined by biuret method using 3-deoxycholate as detergent. The steroids used were dissolved in diethylenglycol (D) and added directly to the incubation medium.

Results. Androgen steroids and their nitrate esters acted on the SuOx activity, in final concentration 10^{-5} *M*/l, according to Figure 1. It can be seen that T and A steroids had a stimulatory effect on the SuOx activity, but their nitrate esters decreased this enzyme activity. Substance B had no effect on the SuOx activity, but its nitrate ester inhibited this enzyme very significantly.

The effect of T on the NCCR activity of liver mitochondria is a linear stimulatory one, between 1.5×10^{-6} and 1.2×10^{-5} *M*/l concentration range (Figure 2), but an inhibited NCCR activity was observed at higher doses of T. TN decreased the NCCR activity in high concentrations. From the Table it is apparent that steroids used in 6×10^{-6} *M*/l concentration had a comparable effect with those of T. The effect of T and A was a stimulatory one (about 50%) and that of B an inhibitory effect (31%). Steroid nitrate esters, exceptly

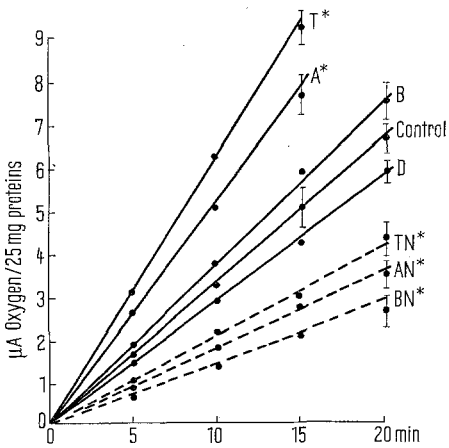

Fig. 1. Action of T, A, B and TN, AN, BN in D solution, and solvent D alone on the SuOx activity of isolated intact rat liver mitochondria incubated in a mixture containing 15 m*M* potassium phosphate buffer (pH 7.4), 1 m*M* EDTA, 0.15 *M* sucrose, 30 m*M* succinate potassium salt and 90 µg cytochrome *c*. Final volume 2 ml. The results obtained are expressed by oxygen consumption in microatoms of oxygen per 25 mg mitochondrial proteins. The significance of the differences ($p < 0.01$) is marked by an asterisk.

Effect of androgen steroids and their nitrate esters on the NCCR activity of isolated 'frozen-thawed' rat liver mitochondria

Steroid	No. of experiments	NAD^+ µ*M*/min per mg protein ± S.E.	± % against control	*p*
Control	20	9.4 ± 0.8	–	–
D	12	8.3 ± 0.8	− 11	> 0.05
T	16	14.5 ± 0.9	+ 52	< 0.01
TN	16	7.5 ± 0.6	− 20	< 0.1
A	16	14.2 ± 1.3	+ 51	< 0.01
AN	16	10.1 ± 0.8	+ 8	> 0.05
B	14	6.5 ± 0.8	− 31	< 0.02
BN	14	5.8 ± 0.9	− 38	< 0.01

Concentration used: 6×10^{-6} *M*/l in solvent D.

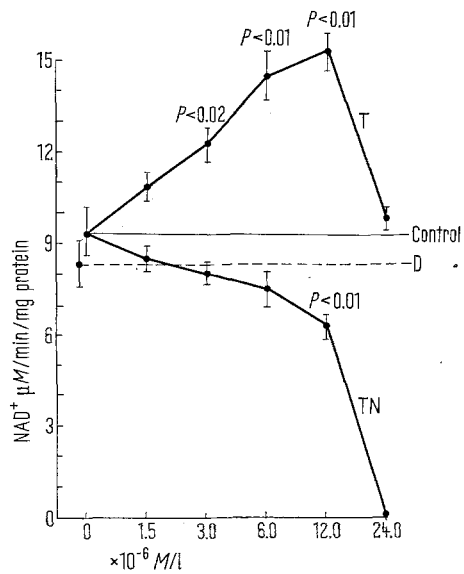

Fig. 2. Action of T and TN depending on the concentration on the NCCR activity of isolated 'frozen-thawed' rat liver mitochondria. The results obtained are expressed in µ*M* NAD^+ per 1 min and 1 mg protein. The significance of the differences is calculated against control values.

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AN, decreased significantly the NCCR activity of liver mitochondria. The solvent D had no effect on the mitochondrial enzymes.

Discussion. Evidence is now accumulating that there is an increase in the rate of energy metabolism of the liver cell upon stimulation by androgen steroids, and that this is reflected in concomitant increase in the biosynthetic activities of liver[6,7]. About the action of androgens upon the mitochondrial electron transport systems, we know very little. Our early experiments revealed a testosterone-induced increase of oxygen consumption and of succinate oxidase activity of rat liver cells, which is evidence of an increased rate of energy metabolism[8].

ENGEL and SCOTT[9], studying the effects of steroid hormones upon the rates of reactions mediated by NADH, showed that testosterone added to a reaction system containing corticosterone blocked the corticosterone-inhibitory effect on glutamate dehydrogenase in beef liver. JENSEN and NEUHARD[10–12] obtained evident inhibitory effect of corticosteroids upon the NADH-cytochrome *c* reductase systems of heart sarcosomes.

We have obtained a stimulatory action of testosterone, in low concentrations, upon the activity of isolated liver mitochondrial oxidative enzyme systems. This action depends on the structure of androgen used, because testosterone and 5α-androstane-17β-ol-3-one showed stimulatory effects, but their nitrate esters had an inverse effect. It can be mentioned that in Enzyme Nomenclature[13] we did not found enzymes with the capacity to hydrolase a steroid nitrate group. We believe that the steroid nitrate esters acted by a mechanism similar to the action of corticosteroids. For the stimulatory action of steroid molecules a 17β-OH and a 3-ketone group is necessary. A double bond at 4-5 position did not influence it. By introducing a nitrate-group into 17β-position, the stimulatory effect of steroid molecule decreased significantly.

Résumé. Le testostérone et la 5α-androstane-17β-ol-3-one stimulent l'activité de la succinoxydase des mitochondries intactes et l'activité de la NADH-cytochrome-*c*-réductase des mitochondries à membranes lésées, extraites du foie du rat blanc. Les nitrates-esters des mêmes stéroïdes réduisent l'activité des deux enzymes.

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Choline Esterase Activity in the Nervous System and the Innervated Organs of the Scorpion, *Heterometrus fulvipes*

Various types of synaptic transmitters occur in the nervous system of arthropods, especially the crustaceans and insects, but too little is known about them in arachnids[1,2]. Acetylcholine was detected in the nervous system of *Limulus polyphemus*[1,3] and the ganglia of the spider, *Heteropoda regia* and 2 species of scorpions, *Buthus europaeus* and *Heterometrus maurus*[4]. Acetylcholine esterase activity was determined in the nervous system and the innervated organs of *Limulus polyphemus*[5]. Small amounts of 5-hydroxytryptamine were also found in the nervous tissue of *Limulus*[6] and the venom apparatus of arachnids[7,8]. Except for these few studies, not much is known about the transmitters in arachnids.

Earlier investigations on the central nervous system of the scorpion[9] showed the occurrence of choline esterase (ChE) in the ventral nerve cord and a diurnal rhythm in its activity. This prompted us to believe that ACh–AChE system might be prevalent in the scorpion as a transmitter system. The present investigation was undertaken to test the validity of the statement.

Material and methods. The commonly available South Indian scorpion, *Heterometrus fulvipes*, was used during the experimentation. The choline esterase activity was determined using the method of Metcalf[10] with due modifications to suit the present material. The incubation mixture contained 0.1 ml of 1% homogenate (W/V) in 0.25 *M* sucrose solution and 1.0 ml of buffer-substrate solution (9 vol. of buffer + 1 vol. of 0.04 *M* acetylcholine chloride solution). 0.008 *M* acetylcholine chloride solution was prepared by mixing 0.04 *M* acetylcholine chloride and the buffer in 1:4 ratio. The mixture was incubated at 37 °C for $^1/_2$ h and the reaction was stopped by adding

Table I. Acetylcholine esterase activity in the nervous system of the scorpion

No.	Tissue	Molar concentration of the substrate 0.004
1	Brain	992.00 – 1292.00 (1143.00 ± 121.80)
2	Suboesophageal mass	1072.00 – 1332.00 (1222.00 ± 131.90)
3	Anterior part of the cord	872.00 – 972.00 (914.5 ± 38.00)
4	Posterior part of the cord	532.00 – 852.00 (759.5 ± 129.00)
5	Segmental nerve	752.00 – 972.00 (889.5 ± 65.20)

Activity expressed as μg of ACh hydrolysed/mg wet wt./h. Values in parentheses are the average of 8 values ± S.D.

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